October 2008

QIAsymphony® DNA Investigator Handbook

QIAsymphony DNA Investigator Kit

For purification of DNA from
surface and buccal swabs
FTA® and Guthrie cards
body fluid stains
chewing gum
cigarette butts
nail clippings and hair
paper and similar materials
small volumes of blood or saliva
bones and teeth
sexual assault specimens
using the QIAsymphony SP

Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

QIAGEN sets standards in:

- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

Our mission is to enable you to achieve outstanding success and breakthroughs. For more information, visit www.qiagen.com.

Contents

Kit Contents **5**
Storage **5**
Product Use Limitations **6**
Product Warranty and Satisfaction Guarantee **6**
Technical Assistance **6**
Quality Control **7**
Safety Information **7**
Introduction **9**
Principle and procedure 9
Description of protocols 11
Equipment and Reagents to Be Supplied by User **12**
Important Notes **14**
Automated purification with the QIAsymphony SP 14
"Reagents and Consumables" drawer 14
"Waste" drawer 16
"Eluate" drawer 16
Inventory scan 17
Starting material 17
Carrier RNA 19
Lysis with proteinase K 19
Storage and quality of purified DNA 19
Quantification of DNA 20
Pretreatment protocols
■ **Pretreatment of Surface and Buccal Swabs** **21**
■ **Pretreatment of FTA and Guthrie Cards** **23**
■ **Pretreatment of Body Fluid Stains** **24**
■ **Pretreatment of Chewing Gum** **26**
■ **Pretreatment of Cigarette Butts** **27**
■ **Pretreatment of Nail Clippings and Hair** **28**
■ **Pretreatment of Paper and Similar Materials** **29**
■ **Pretreatment of Whole Blood or Saliva** **30**

■ **Pretreatment of Bones and Teeth** **31**
■ **Pretreatment of Sexual Assault Specimens** **33**
■ **Pretreatment of Large-Volume Samples** **35**
Purification protocol
■ **Purification of DNA from Casework and Reference Samples** **36**
Troubleshooting Guide **40**
Appendix A: Working with DNA **42**
General handling 42
Disposable plasticware 42
Appendix B: Removing Magnetic-Particle Carryover **42**
References **43**
Ordering Information **44**

Kit Contents

QIAsymphony DNA Investigator Kit	**(192)***
Catalog no.	**931436**
Number of preps	**192**
Reagent Cartridge†‡	2
Enzyme Rack	2
Piercing Lid	2
Buffer ATE‡	20 ml
Buffer AVE‡	20 ml
Buffer ATL	2 x 50 ml
Carrier RNA	310 µg
Proteinase K	3 x 1.4 ml
Reuse Seal Set§	2
Trough Cover (for magnetic particles)	1
Handbook	1

* For 192 x 200 µl preps, 144 x 500 µl preps, or 96 x 1000 µl preps.

† Contains guanidine salts. Not compatible with disinfectants containing bleach. See page 7 for safety information.

‡ Contains sodium azide as a preservative.

§ A Reuse Seal Set contains 8 Reuse Seal Strips.

Storage

QIAsymphony DNA Investigator Kits should be stored at room temperature (15–25°C). Do not store reagent cartridges at temperatures below 15°C.

QIAsymphony DNA Investigator Kits contain ready-to-use proteinase K solution that can be stored at room temperature. To store for extended periods of time, we recommend storing the proteinase K at 2–8°C.

Partially used reagent cartridges can be stored for a maximum of two weeks, enabling cost-efficient reuse of reagents and more flexible sample processing. If a reagent cartridge is partially used, replace the cover of the trough containing the magnetic particles, seal the buffer troughs with the provided Reuse Seal Strips, and close the carrier RNA tubes with screw caps immediately after the end of the protocol run to avoid evaporation.

To avoid evaporation, the reagent cartridge should be open for a maximum of 15 hours (including run times) at a maximum environmental temperature of 30°C.

Running batches with low sample numbers (<24) will increase both the time that the reagent cartridge is open and the required buffer volumes, potentially reducing the total number of sample preparations possible per cartridge.

Product Use Limitations

The QIAsymphony DNA Investigator Kit is intended for molecular biology applications. This product is neither intended for the diagnosis, prevention, or treatment of a disease, nor has it been validated for such use either alone or in combination with other products. Therefore, the performance characteristics of the product for clinical use (i.e., diagnostic, prognostic, therapeutic, or blood banking) is unknown.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

The QIAsymphony DNA Investigator Kit is not intended to be used for isolation and purification of RNA.

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover or visit www.qiagen.com).

Technical Assistance

At QIAGEN, we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced

scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the QIAsymphony DNA Investigator Kit or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please see our Technical Support Center at www.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of QIAsymphony DNA Investigator Kit is tested against predetermined specifications to ensure consistent product quality.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.qiagen.com/Support/MSDS.aspx where you can find, view, and print the MSDS for each QIAGEN kit and kit component.

DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Buffers in the reagent cartridge contain guanidine salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

The following risk and safety phrases apply to components of the QIAsymphony DNA Investigator Kit:

QSL3

Contains guanidine thiocyanate: harmful. Risk and safety phrases:* R20/21/22-32, S13-26-36/37/39-46

QSW1

Contains guanidine hydrochloride and ethanol: highly flammable, harmful, irritant. Risk and safety phrases:* R11-22-36/38, S13-26-36-46

QSW2

Contains ethanol: highly flammable. Risk and safety phrases:* R11, S7-16

Proteinase K

Contains proteinase K: sensitizer, irritant. Risk and safety phrases:* R36/37/38-42/43, S23-24-26-36/37

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

* R11: Highly flammable; R20/21/22: Harmful by inhalation, in contact with skin and if swallowed; R22: Harmful if swallowed; R32: Contact with acids liberates very toxic gas; R36/37/38: Irritating to eyes, respiratory system and skin; R36/38: Irritating to eyes and skin; R42/43: May cause sensitization by inhalation and skin contact; S7: Keep container tightly closed; S13: Keep away from food, drink, and animal feedingstuffs; S16: Keep away from sources of ignition — No smoking; S23: Do not breathe vapor; S24: Avoid contact with the skin; S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; S36/37: Wear suitable protective clothing and gloves; S36/37/39: Wear suitable protective clothing, gloves, and eye/face protection; S46: If swallowed, seek medical advice immediately and show container or label.

Introduction

The QIAsymphony DNA Investigator Kit is designed for automated purification of total DNA from samples encountered in forensic, human identity and biosecurity applications. Proven, performance-leading magnetic-particle technology provides high-quality DNA, which is suitable for direct use in downstream applications, such as quantitative PCR amplification reactions or STR analyses, or for storage for later use. Purified DNA is free of proteins, nucleases, and inhibitors. The QIAsymphony SP performs all steps of the sample extraction procedure after lysis according to the pretreatment protocols. Up to 96 samples are processed in a single run.

Principle and procedure

QIAsymphony technology combines the speed and efficiency of silica-based DNA purification with the convenient handling of magnetic particles (Figure 1). The purification procedure is designed to ensure safe and reproducible handling of precious or potentially infectious samples, and comprises 4 steps: lyse, bind, wash, and elute (see Flowchart, next page). The user can choose different elution volumes between 30 µl to 400 µl of water or modified TE Buffer (Buffer ATE), depending on the protocol. DNA yields depend on sample type, age and storage.

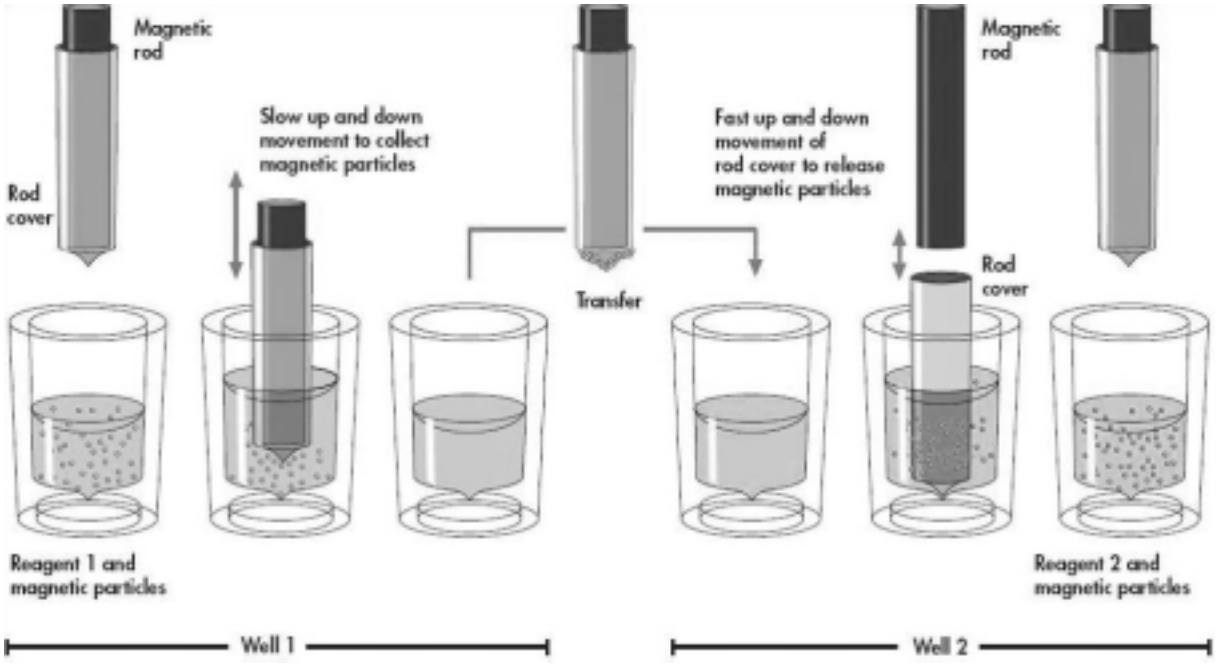
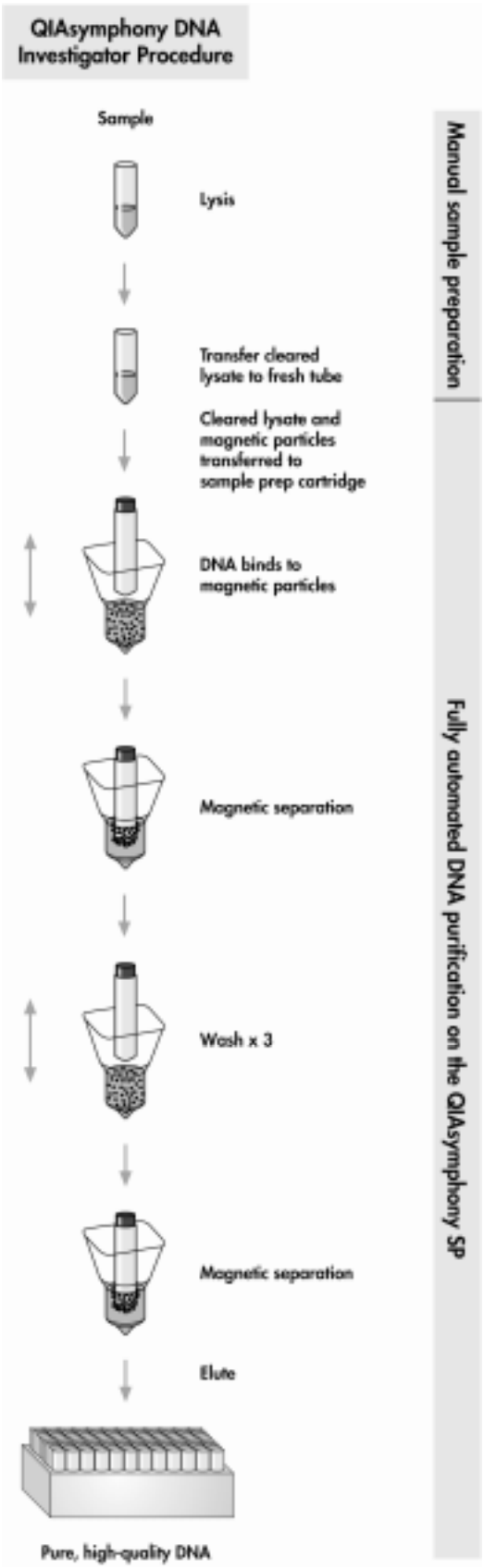

Magnetic rod
Rod cover
Slow up and down movement to collect magnetic particles
Transfer
Fast up and down movement of rod cover to release magnetic particles
Magnetic rod
Rod cover
Reagent 1 and magnetic particles
Reagent 2 and magnetic particles
Well 1
Well 2

Figure 1. Schematic of the QIAsymphony SP principle. The QIAsymphony SP processes a sample containing magnetic particles as follows. A magnetic rod protected by a rod cover enters a well containing the sample and attracts the magnetic particles. The magnetic rod cover is positioned above another well and the magnetic particles are released. The QIAsymphony SP uses a magnetic head containing an array of 24 magnetic rods, and can therefore process up to 24 samples simultaneously. Steps 1 and 2 are repeated several times during sample processing.


QIAsymphony DNA Investigator Procedure
Sample
Lysis
Transfer cleared lysate to fresh tube
Cleared lysate and magnetic particles transferred to sample prep cartridge
DNA binds to magnetic particles
Magnetic separation
Wash x 3
Magnetic separation
Elute
Pure, high-quality DNA
Manual sample preparation
Fully automated DNA purification on the QIAsymphony SP

Description of protocols

This handbook contains two types of protocols.

- Pretreatment protocols detail the preliminary steps, such as proteinase K digestion and removal of solid sample parts, prior to processing on the QIAsymphony SP.
- DNA purification protocols describe setting up the QIAsymphony SP and performing a fully automated run.

Pretreatment protocols

Since the type of samples that can be processed using the QIAsymphony DNA Investigator Kit can vary greatly, there is also a variety of different pretreatments, optimized for specific sample types. Samples are lysed under denaturing conditions in the presence of proteinase K and Buffer ATL in total volumes of either 200 µl, 500 µl, or 1000 µl.

DNA purification protocols

There are two different kinds of DNA purification protocols, which can be used in conjunction with the pretreatment protocols.

- Casework protocols purify genomic and mitochondrial DNA from 200 µl, 500 µl, or 1000 µl of lysate obtained from the sample lysis procedures detailed in the corresponding pretreatment protocols. DNA can be eluted in 30–200 µl of either water or modified TE Buffer (Buffer ATE).
- Reference protocols purify DNA from database samples, such as buccal swabs or dried blood. DNA can be eluted in 100–400 µl of Buffer ATE.

The purified DNA is ready to use in downstream applications.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

All protocols

- Sample Prep Cartridges, 8-well cartridges (cat. no. 997002)
- 8-Rod Covers (cat. no. 997004)
- Filter-Tips, 200 µl and 1500 µl (cat. nos. 990332 and 997024)
- Sample tubes or plates (e.g., 2 ml sample tubes with screw caps, Sarstedt cat. no. 72.693, or without caps, Sarstedt cat. no. 72.608, or S-Blocks, QIAGEN cat. no. 19585). Compatible primary and secondary tube formats are listed at www.qiagen.com/QIAsymphony/Resources
- Elution tubes. Compatible elution tube formats are listed at www.qiagen.com/QIAsymphony/Resources
- Pipets and pipet tips (to prevent cross-contamination, we strongly recommend the use of pipet tips with aerosol barriers)
- Microcentrifuge tubes, 2 ml
- Vortexer
- Thermomixer or shaker–incubator

For swabs

- Plastic swabs with cotton or Dacron® tips (Puritan® applicators with plastic shafts and cotton or Dacron tips are available from: Hardwood Products Company, www.hwppuritan.com, item nos. 25-806 1PC and 25-806 1PD; and from Daigger, www.daigger.com, cat. nos. EF22008D and EF22008DA). Nylon cytology brushes and other swab types may also be used.*
- Microcentrifuge
- Scissors or appropriate cutting device
- Optional: QIAshredder spin columns (for maximum yields), see page 44 for ordering information

* This is not a complete list of suppliers and does not include many important vendors of biological supplies.

For FTA and Guthrie cards

- Filter paper (e.g., QIAcard™ FTA Spots, see page 44 for ordering information)
- Manual paper punch , Harris UNI-CORE, 1.25 mm (cat. no. 159330), 3.00 mm (cat. no. 159331), or equivalent paper punch with cutting mat

For stained material

- Optional: QIAshredder spin columns (for maximum yields), see page 44 for ordering information

For chewing gum, cigarette butts, paper, and similar materials

- Scissors or appropriate cutting device

For nail clippings, hair, and semen stains

- Scissors or appropriate cutting device
- Dithiothreitol (DTT),* 1 M aqueous solution

For bones and teeth

- Metal blender (e.g., Waring†), or TissueLyser II with the Grinding Jar Set, S. Steel, see page 44 for ordering information
- Liquid nitrogen*
- Microcentrifuge

For sexual assault specimens

- Swabs (see above)
- DTT, 1 M aqueous solution
- Additional Buffer ATL and proteinase K, see page 44 for ordering information
- Microcentrifuge

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

† This is not a complete list of suppliers and does not include many important vendors of biological supplies.

Important Notes

Automated purification with the QIAsymphony SP

The QIAsymphony SP makes automated sample preparation easy and convenient. Samples, reagents and consumables, and eluates are separated in different drawers. Simply load samples, reagents provided in special cartridges, and preracked consumables in the appropriate drawer before a run. Start the protocol and remove purified DNA from the "Eluate" drawer after processing. Refer to the *QIAsymphony SP User Manual* for operating instructions.

"Reagents and Consumables" drawer

Reagent cartridges

Reagents for purification of DNA are contained in an innovative reagent cartridge (see Figure 2). Each trough of the reagent cartridge contains a particular reagent, such as magnetic particles, lysis buffer, wash buffer, or elution buffer. Partially used reagent cartridges can be reclosed with Reuse Seal Strips for later reuse, which avoids generation of waste due to leftover reagents at the end of the purification procedure.

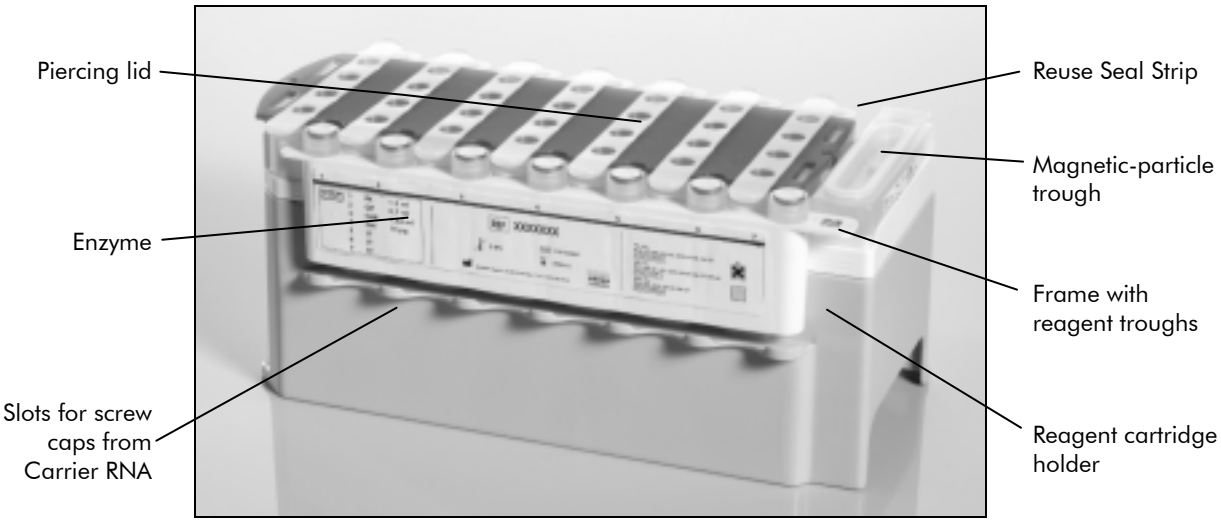


Figure 2. QIAsymphony reagent cartridge. The reagent cartridge contains all reagents required for the protocol run.

Before starting the procedure, ensure that the magnetic particles are fully resuspended. Remove the magnetic-particle trough from the reagent cartridge frame, vortex it vigorously for at least 3 minutes, and replace it in the reagent cartridge frame before the first use. Place the reagent cartridge into the reagent cartridge holder. Place the enzyme rack with the diluted carrier RNA into the reagent cartridge holder. Before using a reagent cartridge for the first time, place the piercing lid on top of the reagent cartridge (Figure 3).

Important: The piercing lid is sharp. Take care when placing it onto the reagent cartridge. Make sure to place the piercing lid onto the reagent cartridge in the correct orientation.

After the magnetic-particle trough cover is removed and the carrier RNA tubes are opened (screw caps can be stored in dedicated slots, see Figure 2), the reagent cartridge is subsequently loaded into the "Reagents and Consumables" drawer.

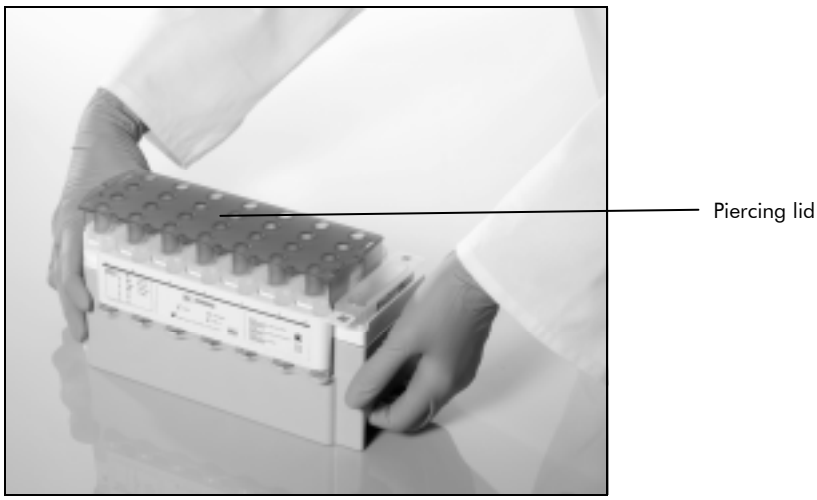


Figure 3. Easy worktable setup with reagent cartridges.

Partially used reagent cartridges can be stored until needed again, see "Storage", page 5.

Loading plasticware

Sample prep cartridges, 8-Rod Covers (both preracked in unit boxes), and disposable filter-tips (200 µl tips provided in blue racks, 1500 µl tips provided in gray racks) are loaded into the "Reagents and Consumables" drawer.

See Table 1(page 17) for the consumables required for QIAsymphony DNA Investigator protocols.

For plasticware ordering information, see page 44.

Note: Both types of tips have filters to help prevent cross-contamination.

Tip rack slots on the QIAsymphony worktable can be filled with either type of tip rack. The QIAsymphony SP will identify the type of tips loaded during the inventory scan.

Note: Do not refill tip racks, unit boxes for sample prep cartridges, or 8-Rod Covers manually before starting another protocol run. The QIAsymphony SP can use partially used tip racks.

"Waste" drawer

Sample prep cartridges and 8-Rod Covers used during a run are re-racked in empty unit boxes in the "Waste" drawer. Make sure that the "Waste" drawer contains sufficient empty unit boxes for plastic waste generated during the protocol run.

Note: Ensure that the covers of the unit boxes are removed before loading the unit boxes into the "Waste" drawer. If you are using 8-Rod Cover boxes for collecting used sample-prep cartridges and 8-Rod Covers, ensure that the box spacer has been removed.

A bag for used filter-tips must be attached to the front side of the "Waste" drawer.

Note: The presence of a tip disposal bag is not checked by the system. Make sure that the tip disposal bag is properly attached before starting a protocol run. For more information, see the *QIAsymphony SP User Manual*.

A waste container collects all liquid waste generated during the purification procedure. The "Waste" drawer can only be closed if the waste container is in place.

"Eluate" drawer

Load the required elution rack into the "Eluate" drawer. Do not load a 96-well plate onto "Elution slot 4". If eluates should be cooled, use "Elution slot 1" with the corresponding cooling adapter. As long-term storage of eluates in the "Eluate" drawer may lead to evaporation of eluates, we strongly recommend using the cooling position.

Inventory scan

Before starting a run, the instrument checks that sufficient consumables for the queued batch(es) have been loaded into the corresponding drawers (Table 1).

Table 1. Consumables required for DNA Investigator protocols

Protocol	Casework 200 µl		Casework 500 µl		Casework 1000 µl		Reference 200 µl		Reference 500 µl	
Number of samples	**24**	**96**	**24**	**96**	**24**	**96**	**24**	**96**	**24**	**96**
Reagent cartridges	1	1	1	2	1	2	1	1	1	2
Sample prep cartridges*	15	60	15	60	15	60	15	60	15	60
8-Rod Covers†	3	12	3	12	3	12	3	12	3	12
1500 µl tips‡	60	216	60	216	60	216	56	200	56	200
200 µl tips‡	30	114	30	114	30	114	26	98	26	98

* 28 sample prep cartridges/unit box.

† Twelve 8-Rod Covers/unit box.

‡ 32 tips/tip rack; numbers include tips for inventory scan.

Starting material

The amount of starting material for use in QIAsymphony DNA Investigator procedures can vary greatly, depending on the amount of DNA in the sample. Specific guidance for starting amounts is given in the individual protocols. The QIAsymphony SP can process 200 µl, 500 µl, or 1000 µl pretreated sample lysates using the casework protocols for purification of DNA or 200 µl or 500 µl pretreated samples lysates using the reference protocols (see "Description of protocols", page 11).

Table 2 and Table 3 provide additional information about protocol options.

Table 2. Starting materials, elution buffers, and DNA purification protocols used in QIAsymphony DNA Investigator procedures

Sample type	Elution buffer	QIAsymphony SP protocol	Assay Control Set*
Surface swabs	Buffer ATE	Casework 500 µl	CW500
	Water	Casework 500 µl	CW500 H2O
Buccal swabs	Buffer ATE	Reference 500 µl	REF500
FTA and Guthrie cards	Buffer ATE	Reference 200 µl	REF200
Body fluid stains	Buffer ATE	Casework 500 µl	CW500
	Water	Casework 500 µl	CW500 H2O
Chewing gum	Buffer ATE	Casework 200 µl	CW200
	Water	Casework 200 µl	CW200 H2O
Cigarette butts	Buffer ATE	Casework 200 µl	CW200
	Water	Casework 200 µl	CW200 H2O
Nail clippings and hair	Buffer ATE	Casework 200 µl	CW200
	Water	Casework 200 µl	CW200 H2O
Paper and similar materials	Buffer ATE	Casework 500 µl	CW500
	Water	Casework 500 µl	CW500 H2O
Blood and saliva, <10 µl	Buffer ATE	Casework 200 µl	CW200
	Water	Casework 200 µl	CW200 H2O
Blood and saliva, 10–50 µl	Buffer ATE	Reference 200 µl	REF200
Bones and teeth	Buffer ATE	Casework 500 µl	CW500
	Water	Casework 500 µl	CW500 H2O
Sexual assault specimen	Buffer ATE	Casework 200 µl	CW200
	Water	Casework 200 µl	CW200 H2O
Large-volume samples	Buffer ATE	Casework 1000 µl	CW1000
	Water	Casework 1000 µl	CW1000 H2O

* The term "Assay Control Set" is used in the user interface when choosing the protocol. For more information about Assay Control Sets, see the *QIAsymphony SP User Manual*.

Table 3. Protocol options for elution and number of samples per kit

Protocol	Elution buffer	Elution volume, µl	Number of samples*
Casework 200 µl	Buffer ATE, Water	30, 40, 50, 100, 150, 200	192
Casework 500 µl	Buffer ATE, Water	30, 40, 50, 100, 150, 200	144
Casework 1000 µl	Buffer ATE, Water	30, 40, 50, 100, 150, 200	96
Reference 200 µl	Buffer ATE	100, 150, 200, 400	192
Reference 500 µl	Buffer ATE	100, 150, 200, 400	144

* Calculations are based on batch sizes of 24 samples. Note that running batches smaller than 24 samples may reduce the number of preparations.

Carrier RNA

The kit is supplied with lyophilized carrier RNA to be used in QIAsymphony DNA Investigator Casework protocols. Carrier RNA enhances binding of DNA to the magnetic beads, especially if there are very few target molecules in the sample. The concentration of carrier RNA allows the procedure to be used as a generic purification system that is compatible with many different amplification systems. Note that eluates contain both carrier RNA and DNA from the sample, with the amount of carrier RNA greatly exceeding the amount of DNA. For details about using carrier RNA, refer to the section "Important points before starting" in the DNA purification protocols.

Lysis with proteinase K

The QIAsymphony DNA Investigator Kit contains proteinase K, which possesses a high specific activity that remains stable over a wide range of temperatures and pH values, with substantially increased activity at higher temperatures. Proteinase K is a recombinant protein expressed in *Pichia pastoris* and is particularly suitable for short digestion times.

Storage and quality of purified DNA

DNA eluted in Buffer ATE or water is immediately ready for use in amplification reactions or can be stored at –80°C, –20°C, or at 2–8°C.

QIAsymphony DNA Investigator procedures yield DNA free of proteins, nucleases, and inhibitors.

Quantification of DNA

Depending on the sample type, the yields of DNA obtained in the purification procedure might be below 1 µg and therefore difficult to quantify using a spectrophotometer. In addition, eluates prepared with carrier RNA might contain much more carrier RNA than target nucleic acids. We recommend using quantitative amplification methods to determine yields.

Carryover of magnetic particles may affect the absorbance reading at 260 nm (A_{260}) of the purified DNA. The measured absorbance at 320 nm (A_{320}) should be subtracted from all absorbance readings. To remove magnetic-particle carryover, see Appendix B, page 42.

Note: For accurate quantification of DNA eluted in Buffer ATE by absorbance at 260 nm, we recommend diluting the sample in elution buffer (Buffer ATE). Dilution of the sample in water may lead to inaccurate values. The elution buffer has a high absorbance at 220 nm, which can lead to high background absorbance levels if the spectrophotometer is not properly zeroed. We therefore strongly recommend using elution buffer as a blank. Extra elution buffer to blank the spectrophotometer is provided in a separate bottle with QIAsymphony DNA Investigator Kits.

Protocol: Pretreatment of Surface and Buccal Swabs

This protocol is for isolation of total (genomic and mitochondrial) DNA from surface, sperm, blood, and saliva swabs. The pretreatment includes lysis of samples using proteinase K.

Important points before starting

- Before beginning the procedure, read "Important Notes", page 14.
- Let the swab air dry for at least 2 h after sample collection.

Things to do before starting

- Set a thermomixer or heated orbital incubator to 56°C for use in step 4.
- If processing semen swabs, prepare an aqueous 1 M DTT* stock solution. Store aliquots at –20°C. Thaw immediately before use.
- If Buffer ATL contains precipitates, dissolve by heating to 70°C with gentle agitation.
- Optional: To harvest lysate remaining in the swab, QIAshredder spin columns may be required.

Procedure

1. **Place the swab into a 2 ml microcentrifuge tube (not provided).**
   If using a Whatman® Omni Swab, eject the swab by pressing the end of the stem towards the swab.

   If using a cotton or Dacron swab, separate the swab from its shaft by hand or using scissors.
2. **Add 475 µl Buffer ATL.**
3. **Add 25 µl proteinase K, and mix by vortexing. If processing semen swabs, add 20 µl 1 M DTT.**
4. **Place the tube in a thermomixer or heated orbital incubator, and incubate with shaking at 900 rpm at 56°C for 15 min.**
5. **Briefly centrifuge the tube in a microcentrifuge to remove drops from the inside of the lid.**

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

6. **Carefully transfer the lysate to sample tubes or plates that are compatible with the sample rack of the QIAsymphony SP.**

   See www.qiagen.com/QIAsymphony/Resources for a full list of compatible vessels. We recommend using 2 ml tubes (e.g. Sarstedt, cat. no. 72.693 or 72.608) or S-Blocks (see ordering information, page 44).

   Note: Do not transfer any solid material as this may clog the tips during automated DNA purification.

   Lysate remaining in the swab can be harvested by transferring the material to a QIAshredder spin column (not supplied) and centrifuging at full speed for 2 min in a microcentrifuge. Transfer the flow-through to the sample tube.

7. **Continue with the protocol "DNA Purification from Casework and Reference Samples" (page 36).**

Protocol: Pretreatment of FTA and Guthrie Cards

This protocol is for isolation of total (genomic and mitochondrial) DNA from whole blood, saliva, or buccal cells dried and immobilized on FTA cards, Guthrie cards, or similar collection devices. The pretreatment includes lysis of samples using proteinase K.

Important point before starting

- Before beginning the procedure, read "Important Notes", page 14.

Things to do before starting

- Set a thermomixer or heated orbital incubator to 56°C for use in step 4.
- If Buffer ATL contains precipitates, dissolve by heating to 70°C with gentle agitation.

Procedure

1. **Cut punches from a dried spot with a single-hole paper punch. Place 1 x 1.2 mm, 2 x 1.2 mm, or 1 x 3 mm diameter card punch(es) into a 2 ml microcentrifuge tube (not provided).**
2. **Add 180 µl Buffer ATL.**
3. **Add 20 µl of proteinase K, and mix by vortexing.**
4. **Place the tube in a thermomixer or heated orbital incubator, and incubate with shaking at 900 rpm at 56°C for 15 min.**
5. **Carefully transfer the lysate to sample tubes or plates that are compatible with the sample rack of the QIAsymphony SP.**

   See www.qiagen.com/QIAsymphony/Resources for a full list of compatible vessels. We recommend using 2 ml tubes (e.g. Sarstedt, cat. no. 72.693 or 72.608) or S-Blocks (see ordering information, page 44).

   Note: Do not transfer any solid material as this may clog the tips during automated DNA purification.
6. **Continue with the protocol "DNA Purification from Casework and Reference Samples" (page 36).**

Protocol: Pretreatment of Body Fluid Stains

This protocol is for isolation of total (genomic and mitochondrial) DNA from material stained with blood, saliva, or semen. The pretreatment includes lysis of samples using proteinase K. For samples requiring larger lysis volumes, see the "Pretreatment of Large-volume Samples" protocol (page 35).

Important point before starting

- Before beginning the procedure, read "Important Notes", page 14.

Things to do before starting

- Set a thermomixer or heated orbital incubator to 56°C for use in step 4.
- If processing semen stains, prepare an aqueous 1 M DTT* stock solution. Store aliquots at –20°C. Thaw immediately before use.
- If Buffer ATL contains precipitates, dissolve by heating to 70°C with gentle agitation.
- Optional: To harvest lysate remaining in the stained material, QIAshredder spin columns may be required.

Procedure

1. **Place the stained material into a 2 ml microcentrifuge tube (not provided).**
2. **Add 475 µl Buffer ATL.**
3. **Add 25 µl proteinase K and mix by vortexing. If processing semen stains, add 20 µl 1 M DTT.**
4. **Place the tube in a thermomixer or heated orbital incubator, and incubate with shaking at 900 rpm at 56°C for 15 min.**

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

5. **Carefully transfer the lysate to sample tubes or plates that are compatible with the sample rack of the QIAsymphony SP.**

   See www.qiagen.com/QIAsymphony/Resources for a full list of compatible vessels. We recommend using 2 ml tubes (e.g. Sarstedt, cat. no. 72.693 or 72.608) or S-Blocks (see ordering information, page 44).

   Note: Do not transfer any solid material as this may clog the tips during automated DNA purification.

   Lysate remaining in solid sample material (e.g., denim) can be harvested by transferring the material to a QIAshredder spin column (not supplied) and centrifuging at full speed for 2 min in a microcentrifuge. Transfer the flow-through to the sample tube.

6. **Continue with the protocol "DNA Purification from Casework and Reference Samples" (page 36).**

Protocol: Pretreatment of Chewing Gum

This protocol is for isolation of total (genomic and mitochondrial) DNA from chewing gum. The pretreatment includes lysis of samples using proteinase K.

Important point before starting

- Before beginning the procedure, read "Important Notes", page 14.

Things to do before starting

- Set a thermomixer or heated orbital incubator to 56°C for use in step 4.
- If Buffer ATL contains precipitates, dissolve by heating to 70°C with gentle agitation.

Procedure

1. **Cut up to 30 mg of chewing gum into small pieces and transfer them to a 1.5 ml or 2 ml microcentrifuge tube (not provided).**
2. **Add 180 µl Buffer ATL.**
3. **Add 20 µl of proteinase K, and mix by vortexing.**
4. **Place the tube in a thermomixer or heated orbital incubator, and incubate with shaking at 900 rpm at 56°C for 15 min.**
5. **Carefully transfer the lysate to sample tubes or plates that are compatible with the sample rack of the QIAsymphony SP.**

   See www.qiagen.com/QIAsymphony/Resources for a full list of compatible vessels. We recommend using 2 ml tubes (e.g. Sarstedt, cat. no. 72.693 or 72.608) or S-Blocks (see ordering information, page 44).

   Note: Do not transfer any solid material as this may clog the tips during automated DNA purification.
6. **Continue with the protocol "DNA Purification from Casework and Reference Samples" (page 36).**

Protocol: Pretreatment of Cigarette Butts

This protocol is for isolation of total (genomic and mitochondrial) DNA from cigarette butts. The pretreatment includes lysis of samples using proteinase K.

Important point before starting

- Before beginning the procedure, read "Important Notes", page 14.

Things to do before starting

- Set a thermomixer or heated orbital incubator to 56°C for use in step 4.
- If Buffer ATL contains precipitates, dissolve by heating to 70°C with gentle agitation.

Procedure

1. **Remove 1 cm^2 of the outer paper from the end of the cigarette or filter. Cut the piece into six smaller pieces and transfer to a 1.5 ml or 2 ml microcentrifuge tube (not provided).**
2. **Add 180 µl Buffer ATL.**
3. **Add 20 µl of proteinase K, and mix by vortexing.**
4. **Place the tube in a thermomixer or heated orbital incubator, and incubate with shaking at 900 rpm at 56°C for 15 min.**
5. **Carefully transfer the lysate to sample tubes or plates that are compatible with the sample rack of the QIAsymphony SP.**

   See www.qiagen.com/QIAsymphony/Resources for a full list of compatible vessels. We recommend using 2 ml tubes (e.g. Sarstedt, cat. no. 72.693 or 72.608) or S-Blocks (see ordering information, page 44).

   Note: Do not transfer any solid material as this may clog the tips during automated DNA purification.
6. **Continue with the protocol "DNA Purification from Casework and Reference Samples" (page 36).**

Protocol: Pretreatment of Nail Clippings and Hair

This protocol is for isolation of total (genomic and mitochondrial) DNA from nail clippings or hair. The pretreatment includes lysis of samples using proteinase K and DTT*.

Important point before starting

- Before beginning the procedure, read "Important Notes", page 14.

Things to do before starting

- Set a thermomixer or heated orbital incubator to 56°C for use in step 4.
- Prepare an aqueous 1 M DTT stock solution. Store aliquots at –20°C. Thaw immediately before use.
- If Buffer ATL contains precipitates, dissolve by heating to 70°C with gentle agitation.

Procedure

1. **Cut nail clippings or hairs to 0.5–1 cm lengths, and transfer to a 1.5 ml or 2 ml microcentrifuge tube (not provided).**
2. **Add 160 µl Buffer ATL.**
3. **Add 20 µl proteinase K and 20 µl 1 M DTT. Mix by vortexing.**
4. **Place the tube in a thermomixer or heated orbital incubator, and incubate with shaking at 900 rpm at 56°C for 1 h.**

   In general, samples are lysed in 1 h. If necessary, increase the incubation time to ensure complete lysis.
5. **Carefully transfer the lysate to sample tubes or plates that are compatible with the sample rack of the QIAsymphony SP.**

   See www.qiagen.com/QIAsymphony/Resources for a full list of compatible vessels. We recommend using 2 ml tubes (e.g. Sarstedt, cat. no. 72.693 or 72.608) or S-Blocks (see ordering information, page 44).

   Note: Do not transfer any solid material as this may clog the tips during automated DNA purification.
6. **Continue with the protocol "DNA Purification from Casework and Reference Samples" (page 36).**

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Protocol: Pretreatment of Paper and Similar Materials

This protocol is for isolation of total (genomic and mitochondrial) DNA from paper evidence samples, such as saliva on envelope flaps and stamps or fingerprints on documents. The pretreatment includes lysis of samples using proteinase K. For samples requiring larger lysis volumes, see the "Pretreatment of Large-Volume Samples" protocol (page 35).

Important point before starting

- Before beginning the procedure, read "Important Notes", page 14.

Things to do before starting

- Set a thermomixer or heated orbital incubator to 56°C for use in step 4.
- If Buffer ATL contains precipitates, dissolve by heating to 70°C with gentle agitation.

Procedure

1. **Remove a 0.5 to 2.5 cm^2 piece from the paper or similar material, and cut into smaller pieces. Transfer the pieces to a 2 ml microcentrifuge tube (not provided).**
2. **Add 475 µl Buffer ATL.**
3. **Add 25 µl of proteinase K, and mix by vortexing.**
4. **Place the tube in a thermomixer or heated orbital incubator, and incubate with shaking at 900 rpm at 56°C for 15 min.**
5. **Carefully transfer the lysate to sample tubes or plates that are compatible with the sample rack of the QIAsymphony SP.**

   See www.qiagen.com/QIAsymphony/Resources for a full list of compatible vessels. We recommend using 2 ml tubes (e.g. Sarstedt, cat. no. 72.693 or 72.608) or S-Blocks (see ordering information, page 44).

   Note: Do not transfer any solid material as this may clog the tips during automated DNA purification.
6. **Continue with the protocol "DNA Purification from Casework and Reference Samples" (page 36).**

Protocol: Pretreatment of Whole Blood or Saliva

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from up to 50 µl of whole blood treated with EDTA,* citrate,* or heparin-based* anticoagulants or up to 50 µl of saliva. The pretreatment includes lysis of samples using proteinase K.

Important point before starting

- Before beginning the procedure, read "Important Notes", page 14.

Thing to do before starting

- Equilibrate samples to room temperature (15–25°C).
- Set a thermomixer or heated orbital incubator to 56°C for use in step 4.
- If Buffer ATL contains precipitates, dissolve by heating to 70°C with gentle agitation.

Procedure

1. **Pipet up to 50 µl blood or saliva into a sample tube or plate that is compatible with the sample rack of the QIAsymphony SP.**
   See www.qiagen.com/QIAsymphony/Resources for a full list of compatible vessels. We recommend using 2 ml tubes (e.g. Sarstedt, cat. no. 72.693 or 72.608) or S-Blocks (see ordering information, page 44).
2. **Add Buffer ATL to a final volume of 180 µl.**
3. **Add 20 µl of proteinase K, and mix by vortexing.**
4. **Place the tube or plate into a thermomixer or heated orbital incubator and incubate with shaking at 900 rpm at 56°C for 10 min.**
5. **Continue with the protocol "DNA Purification from Casework and Reference Samples" (page 36).**

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Protocol: Pretreatment of Bones and Teeth

This protocol is for isolation of total (genomic and mitochondrial) DNA from pieces of bones and teeth. The pretreatment includes the mechanical disruption and lysis of samples.

Important points before starting

- Before beginning the procedure, read "Important Notes", page 14.
- Lysis time will vary depending on the size and density of the source material. The lysis conditions given here are intended to serve as guidelines.

Things to do before starting

- Set a thermomixer or heated orbital incubator to 56°C for use in step 5.
- If Buffer ATL contains precipitates, dissolve by heating to 70°C with gentle agitation.

Procedure

1. **Crush the bone or teeth into small fragments. Grind to a fine powder using a metal blender half-filled with liquid nitrogen.* Alternatively, grind the bone or teeth to a fine powder using the TissueLyser II and the Grinding Jar Set, S. Steel.**

   When using the TissueLyser II, transfer the bone or tooth sample and the ball into the grinding jar. Pour liquid nitrogen into the grinding jar over the ball and bone or tooth fragments. Allow the temperature to equilibrate (i.e., liquid nitrogen stops boiling). Decant the excess liquid nitrogen, close the grinding jar with the lid, and transfer it to the TissueLyser II. Grind the bone at 30 Hz for 1 min or until the sample is pulverized (grinding times depend on type, condition, and size of sample).
2. **Transfer up to100 mg of bone or teeth powder in a 1.5 ml or 2 ml microcentrifuge tube (not provided).**
3. **Add 475 µl Buffer ATL.**
4. **Add 25 µl of proteinase K, and mix by vortexing.**
5. **Place the tube in a thermomixer or heated orbital incubator, and incubate with shaking at 900 rpm at 56°C overnight.**
6. **Centrifuge the tube at full speed for 1 min in a microcentrifuge.**

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

7. **Carefully transfer the lysate to sample tubes or plates that are compatible with the sample rack of the QIAsymphony SP.**

   See www.qiagen.com/QIAsymphony/Resources for a full list of compatible vessels. We recommend using 2 ml tubes (e.g. Sarstedt, cat. no. 72.693 or 72.608) or S-Blocks (see ordering information, page 44).

   Note: Do not transfer any solid material as this may clog the tips during automated DNA purification.

8. **Continue with the protocol "DNA Purification from Casework and Reference Samples" (page 36).**

Protocol: Pretreatment of Sexual Assault Specimens

This protocol is for differential extraction of total (genomic and mitochondrial) DNA from fabrics or swabs containing epithelial cells mixed with sperm cells. The pretreatment includes differential lysis of epithelial and sperm cells.

Important point before starting

- Before beginning the procedure, read "Important Notes", page 14.

Things to do before starting

- Set a thermomixer or heated orbital incubator to 56°C for use in steps 4 and 10.
- Prepare an aqueous 1 M DTT* stock solution. Store aliquots at –20°C. Thaw immediately before use.
- If Buffer ATL contains precipitates, dissolve by heating to 70°C with gentle agitation.
- Additional Buffer ATL and proteinase K is required for this protocol and can be ordered separately (see ordering information, page 44)
- Optional: To harvest lysate remaining in the swab or stain, QIAshredder spin columns may be required.

Procedure

1. **Place the swab or the piece of fabric into a 2 ml microcentrifuge tube (not provided).**

   If using an Omni Swab, eject the swab by pressing the end of the stem towards the swab.

   If using a cotton or Dacron swab, separate the swab from its shaft by hand or using scissors.

2. **Add 475 µl Buffer ATL.**
3. **Add 25 µl of proteinase K, and mix by vortexing.**
4. **Place the tube in a thermomixer or heated orbital incubator, and incubate with shaking at 900 rpm at 56°C for 1 h.**

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

5. **Remove the solid material from the tube.**

   Lysate remaining in the swab or fabric can be harvested by transferring the material to a QIAshredder spin column (not supplied) and centrifuging at full speed for 2 min in a microcentrifuge. Remove and discard the QIAshredder column and the solid material.

6. **Centrifuge the tube for 5 min at full speed in a microcentrifuge. Carefully transfer all but 30 µl of the supernatant to a new tube without disturbing the pellet.**

   Note: For isolation of DNA from epithelial cells, transfer 200 µl of the supernatant into an appropriate sample tube and continue with the protocol "DNA Purification from Casework and Reference Samples" (page 36).

7. **Resuspend the pellet in 500 µl Buffer ATL. Close the lid and mix by pulse-vortexing for 10 s. Centrifuge the tube for 5 min at full speed in a microcentrifuge. Carefully aspirate and discard all but 30 µl of the supernatant without disturbing the pellet.**

8. **Repeat step 7 for a total of at least 4 times.**

   Note: The ratio of epithelial cells to sperm cells influences the number of repeats needed for purification of sperm nuclei.

9. **Add 160 µl Buffer ATL, 20 µl proteinase K, and 20 µl 1 M DTT to the pellet. Mix by vortexing.**

10. **Place the tube in a thermomixer or heated orbital incubator, and incubate with shaking at 900 rpm at 56°C for at least 1 h.**

11. **Carefully transfer the lysate to sample tubes or plates that are compatible with the sample rack of the QIAsymphony SP.**

    See www.qiagen.com/QIAsymphony/Resources for a full list of compatible vessels. We recommend using 2 ml tubes (e.g. Sarstedt, cat. no. 72.693 or 72.608) or S-Blocks (see ordering information, page 44).

    Note: Do not transfer any solid material as this may clog the tips during automated DNA purification.

12. **Continue with the protocol "DNA Purification from Casework and Reference Samples" (page 36).**

Protocol: Pretreatment of Large-Volume Samples

This protocol is for isolation of total (genomic and mitochondrial) DNA from forensic samples that require increased volumes for thorough lysis, e.g., diffuse stains on fabric or paper. The pretreatment includes lysis of samples using proteinase K.

Important point before starting

- Before beginning the procedure, read "Important Notes", page 14.

Things to do before starting

- Set a thermomixer or heated orbital incubator to 56°C for use in step 4.
- If Buffer ATL contains precipitates, dissolve by heating to 70°C with gentle agitation.

Procedure

1. **Transfer samples to a 2 ml microcentrifuge tube (not provided). Large samples can be cut into smaller pieces to fit more conveniently.**
2. **Add 960 µl Buffer ATL.**
3. **Add 40 µl of proteinase K and mix by vortexing. If processing semen samples, add 40 µl 1 M DTT.**
4. **Place the tube in a thermomixer or heated orbital incubator, and incubate with shaking at 900 rpm at 56°C for at least 15 min.**
5. **Carefully transfer the lysate to sample tubes or plates that are compatible with the sample rack of the QIAsymphony SP.**

   See www.qiagen.com/QIAsymphony/Resources for a full list of compatible vessels. We recommend using 2 ml tubes (e.g. Sarstedt, cat. no. 72.693 or 72.608) or S-Blocks (see ordering information, page 44).

   Note: Do not transfer any solid material as this may clog the tips during automated DNA purification.

   Lysate remaining in solid sample material (e.g., denim) can be harvested by transferring the material to a QIAshredder spin column (not supplied) and centrifuging at full speed for 2 min in a microcentrifuge. Transfer the flow-through to the sample tube or plate.
6. **Continue with the protocol "DNA Purification from Casework and Reference Samples" (page 36).**

Protocol: Purification of DNA from Casework and Reference Samples

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from various forensic casework and reference samples that have been prepared as described in the pretreatment protocols in this handbook (pages 21–35). The protocol describes the procedure for setting up the QIAsymphony SP and starting a run. See Tables 2 and 3, page 18 and 19, for a summary of protocol options.

Casework protocols are designed to ensure efficient extraction of DNA from a wide range of demanding samples. The fully automated procedure processes sample lysate volumes of 200 µl, 500 µl, or 1000 µl. DNA is eluted in 30–200 µl of either buffer ATE or water. Carrier RNA is added to the samples during the automated procedure to maximize yields from very small samples.

Reference protocols allow robust and time-optimized extraction of database samples. DNA is eluted in 100–400 µl Buffer ATE.

Important points before starting

- Ensure that you are familiar with operating the QIAsymphony SP. Refer to the *QIAsymphony SP User Manual* for operating instructions.
- Before beginning the procedure, read "Important Notes" starting on page 14.
- Try to avoid vigorous shaking of the reagent cartridge otherwise foam may be generated, which can lead to liquid-level detection problems.
- Before using a reagent cartridge for the first time, check that Buffer QSL3 does not contain a precipitate. If necessary, remove the trough containing Buffer QSL3 from the reagent cartridge and incubate for 30 minutes at 37°C with occasional shaking to dissolve precipitate. Make sure to replace the trough in the correct position. If the reagent cartridge is already pierced, make sure that the troughs are sealed with Reuse Seal Strips and incubate the complete reagent cartridge for 30 minutes at 37°C with occasional shaking in a water bath.

Things to do before starting

- Remove any solid material from the sample tube. Note that there is no need to replace lysate volumes absorbed into the material. Optional: QIAshredder spin columns can be used to harvest lysate remaining in absorbent materials. See page 44 for ordering information.
- Dissolve the lyophilized carrier RNA in 1.6 ml Buffer ATE (provided in the QIAsymphony DNA Investigator Kit) before using the kit for the first time. Transfer 400 µl to each of the tubes in positions 1 and 2 of the enzyme rack on the reagent cartridge. Add additional 1.2 ml Buffer ATE to each tube and mix by pipetting up and down several times.

  Note: It is important that the final volume of carrier RNA in the tubes of the enzyme rack is exactly 1.6 ml. Dissolved carrier RNA is stable for 4 weeks when stored at 2–8°C. For longer periods, store carrier RNA at –20°C.

  Note: For the inventory scan to be completed successfully, tubes containing carrier RNA must be opened and placed in the enzyme rack which is placed in the reagent cartridge. The carrier RNA, however, will not be used for reference protocols.
- Before starting the procedure, ensure that the magnetic particles are fully resuspended. Vortex the trough containing the magnetic particles vigorously for at least 3 minutes before first use.
- Before loading the reagent cartridge remove the cover from the trough containing the magnetic particles and open the carrier RNA tubes. Make sure that the piercing lid is placed on the reagent cartridge or, if using a partially used reagent cartridge, make sure the Reuse Seal Strips have been removed.
- If samples are bar coded, orient samples in the tube carrier so that the bar codes face the bar code reader at the left side of the QIAsymphony SP.
- See www.qiagen.com/QIAsymphony/Resources for more information about compatible vessels.

Procedure

1. **Ensure that the QIAsymphony SP is switched on.**
   The power switch is located at the bottom, left corner of the QIAsymphony SP.
2. **Ensure the "Waste" drawer is prepared properly, and perform an inventory scan of the "Waste" drawer, including the tip chute and liquid waste. Replace the tip disposal bag if necessary.**
3. **Load the required reagent cartridge(s) and consumables (see Table 1, page 17) into the "Reagents and Consumables" drawer, and perform an inventory scan of the "Reagents and Consumables" drawer.**

4. **Load the required elution rack into the "Eluate" drawer.**

   Do not load a 96-well plate onto "Elution slot 4".

   If eluates should be cooled, use "Elution slot 1" with the corresponding cooling adapter.

5. **Place the samples into the appropriate sample carrier, and load them into the "Sample" drawer.**

6. **Using the touchscreen, enter the required information for each batch of samples to be processed.**

   Enter the following information:

   - Sample information (depending on sample racks used)
   - Protocol ("Assay control set") to be run
   - Elution volume and output position

   After information about the batch has been entered, the status changes from "LOADED" to "QUEUED". As soon as one batch is queued the "Run" button appears.

7. **Press the "Run" button to start processing.**

   All processing steps are fully automated. At the end of the protocol run, the status of the batch changes from "RUNNING" to "COMPLETED".

8. **Retrieve the elution rack containing the purified DNA from the "Eluate" drawer.**

   The DNA is ready to use, or can be stored at 2–8°C, –20°C, or –80°C.

   In general, magnetic particles are not carried over into eluates. If carryover does occur, magnetic particles in eluates will not affect most downstream applications. If magnetic particles need to be removed before performing downstream applications, tubes or plates containing eluates should first be placed in a suitable magnet and the eluates transferred to a clean tube (see Appendix B, page 42).

   If the eluate drawer is closed when a batch is running (e.g., if elution racks which contain the eluates are removed), the run will be paused and an inventory scan of the "Eluate" drawer will be performed. A message appears during the scan and must be closed (by pressing the "Close" button) before the run can be restarted.

   Result files are generated for each elution plate.

9. **If the reagent cartridge(s) is only partially used, seal it with the provided Reuse Seal Strips and close the carrier RNA tubes with screw caps immediately after the end of the protocol run to avoid evaporation.**

   Note: For more information about storage of partially used reagent cartridges, see "Storage" on page 5.

10. **Discard used sample tubes, plates, and waste according to your local safety regulations.**

    See page 7 for safety information.

11. **Clean the QIAsymphony SP.**

    Follow the maintenance instructions in the *QIAsymphony SP User Manual*.

12. **Close the workstation drawers, and switch off the QIAsymphony SP.**

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

	Comments and suggestions
General handling	
Error message displayed in the touch screen	If an error message is displayed in the touchscreen during a protocol run, refer to "Troubleshooting" in the *QIAsymphony SP User Manual*.
Precipitate in reagent trough of opened cartridge	
a) Buffer evaporation	Excessive evaporation can lead to increased salt concentration in buffers. Discard reagent cartridge. Make sure to seal buffer troughs of partially used reagent cartridge when not being used for DNA purification.
b) Storage of reagent cartridge	Storage of reagent cartridge under 15°C may lead to the formation of precipitates. If necessary, remove the trough containing Buffer QSL3 from the reagent cartridge and incubate for 30 min at 37°C with occasional shaking to dissolve the precipitate. If the reagent cartridge is already pierced, make sure that the reagent cartridge is reclosed with the Reuse Seal Set and incubate the complete reagent cartridge for 30 min at 37°C with occasional shaking in a water bath.

	Comments and suggestions
Low DNA yield	
a) Magnetic particles were not completely resuspended	Before starting the procedure, ensure that the magnetic particles are fully resuspended. Vortex for at least 3 min before use.
b) Incomplete lysis of samples	Proteinase K was stored at high temperatures for a prolonged time. Repeat the procedure using new samples and fresh proteinase K.
c) Clogging of pipet tip due to insoluble material	Solid sample material was not removed from the digested sample prior to starting the QIAsymphony DNA purification procedure.
DNA does not perform well in downstream applications	
a) Insufficient DNA used in downstream application	See "Low DNA yield" (page 40) for possible reasons. If possible, increase the amount of eluate used in the reaction.
b) Reduced sensitivity	Determine the maximum volume of eluate suitable for your amplification reaction. Reduce or increase the volume of eluate added to the reaction accordingly.

Appendix A: Working with DNA

General handling

Proper microbiological aseptic technique should always be used when working with small sample sizes. Hands and dust particles may carry bacteria and molds, and are the most common sources of contamination. Always wear latex or vinyl gloves while handling reagents and samples to prevent contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed.

Disposable plasticware

The use of sterile, disposable polypropylene tubes is recommended throughout the purification procedure. These tubes are generally DNase-free.

Appendix B: Removing Magnetic-Particle Carryover

In general, magnetic particles are not carried over into eluates. If carryover does occur, magnetic particles in eluates will not affect most downstream applications.

Carryover of magnetic particles in the eluate may affect the absorbance at 260 nm (A_{260}) in a spectrophotometer. To remove particles, the tube containing the eluate should first be applied to a suitable magnetic separator and the eluate transferred to a clean tube:

- Apply the tube containing the nucleic acids to a suitable magnetic separator (e.g., QIAGEN 12-Tube Magnet, cat. no. 36912) until the magnetic particles are separated. If eluate is in microplates, apply the microplate to a suitable magnetic separator (e.g., QIAGEN 96-Well Magnet Type A, cat. no. 36915) until the magnetic particles are separated.
- If a suitable magnetic separator is not available, centrifuge the tube containing the DNA for 1 minute at full speed in a microcentrifuge to pellet any remaining magnetic particles.
- Once separation is complete, carefully withdraw an aliquot for quantification and dilute as necessary.
- Measure the absorbance at 320, 280, and 260 nm. Subtract the absorbance reading obtained at 320 nm from the readings obtained at 260 and 280 nm to correct for the presence of magnetic particles.

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen.com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

Ordering Information

Product	Contents	Cat. no.
QIAsymphony DNA Investigator Kit (192)	For 192 x 200 µl preps, 144 x 500 µl preps, or 96 x 1000 µl preps.: Includes 2 reagent cartridges enzyme racks and accessories	931436
Related products		
Accessory Trough (10)	Accessory troughs for use with the QIAsymphony SP	997012
Reagent Cartridge Holder (2)	Reagent cartridge holder for use with the QIAsymphony SP	997008
Sample Carrier, plate, Qsym	Plate carrier for sample input. For use with the QIAsymphony SP	9017660
Tube Insert, 11 mm, sample carrier, Qsym	Primary tube adapter (11 mm) for use with the QIAsymphony tube carrier	9241033
Tube Insert, 13 mm, sample carrier, Qsym	Primary tube adapter (13 mm) for use with the QIAsymphony tube carrier	9241034
Tube Insert, 2 ml, sample carrier, Qsym	Secondary tube adapter (for 2 ml screw-cap tubes) for use with the QIAsymphony tube carrier	9241032
Cooling Adapter, tubes, 2 ml, Qsym	Cooling adapter for 2 ml screw-cap tubes. For use in the QIAsymphony "Eluate" drawer	9018088
Cooling Adapter, EMT, Qsym	Cooling adapter for EMT racks. For use in the QIAsymphony "Eluate" drawer	9018086
Cooling Adapter, MTP, RB, Qsym	Cooling adapter for round bottom microtiter plates (MTP). For use in the QIAsymphony "Eluate" drawer	9018085
Cooling Adapter, PCR, Qsym	Cooling adapter for PCR plates. For use in the QIAsymphony "Eluate" drawer	9018087
Adapter, tubes, 2 ml, Qsym	Adapter for 2 ml screw-cap tubes. For use in the QIAsymphony "Eluate" drawer	9018577

Product	Contents	Cat. no.
Sample Prep Cartridges, 8-well (336)	8-well sample prep cartridges for use with the QIAsymphony SP	997002
8-Rod Covers (144)	8-Rod Covers for use with the QIAsymphony SP	997004
Filter-Tips, 200 µl (1024)	Disposable Filter-Tips, racked; (8 x 128). For use with the QIAcube and the QIAsymphony SP	990332
Filter-Tips, 1500 µl (1024)	Disposable Filter-Tips, racked; (8 x 128). For use with the QIAsymphony SP	997024
Tip Disposable Bags (15)	Tip disposal bags for use with the QIAsymphony SP	9013395
Buffer ATL (200 ml)	200 ml Tissue Lysis Buffer for 1000 preps	19076
QIAGEN Proteinase K (2 ml)	For protease digestion during DNA and RNA preparation. Contents: 2 ml (>600 mAU/ml, solution)	19131
QIAshredder (50)	50 disposable cell-lysate homogenizers for use in nucleic acid minipreps, caps	79654
TissueLyser II	Universal laboratory mixer-mill disruptor, 100–120/220–240 V 50/60 Hz	85300
TissueLyser Adapter Set 2 x 24	2 sets of Adapter Plates and 2 racks for use with 2 ml microcentrifuge tubes on the TissueLyser II	69982
Stainless Steel Beads, 5 mm (200)	Stainless Steel Beads, suitable for use with the TissueLyser system	69989
QIAcard FTA One Spot (100)	For 100 samples: 100 QIAcard FTA One Spots	159201
QIAcard FTA Two Spots (100)	For 100 x 2 samples: 100 QIAcard FTA Two Spots	159203
QIAcard FTA Four Spots (100)	For 100 x 4 samples: 100 QIAcard FTA Four Spots	159205

Product	Contents	Cat. no.
QIAcard FTA Indicator Four Spots (25)	For 25 x 4 samples: 25 QIAcard FTA Indicator Four Spots	159214
12-Tube Magnet	Magnet for separating magnetic particles in 12 x 1.5 ml or 2 ml tubes	36912
96-Well Magnet Type A	Magnet for separating magnetic particles in wells of 96-well plates, 2 x 96-Well Microplates FB	36915
S-Blocks (24)	96-well blocks with 2.2 ml wells, 24 per case	19585

The TissueLyser II and QIAcard FTA Spots are intended for molecular biology applications. These products are neither intended for the diagnosis, prevention, or treatment of a disease, nor have they been validated for such use either alone or in combination with other products.

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